

WHAT IS CLAIMED IS:

1. A method of analysing the methylation state of one or more nucleotide sequences comprising the steps of:

a) selecting one or more genomic test nucleotide sequences from one or more subjects that exhibit a phenotype of interest, and one or more corresponding genomic control sequences from one or more control subjects that lack the phenotype of interest;

b) digesting the genomic test nucleotide sequences and the separately digesting genomic control sequences with one or more methylation-sensitive restriction endonucleases, to produce ends that can be ligated to an adaptor nucleotide sequence;

c) ligating adaptor nucleotide sequences to the ends produced from step b) to produce ligated sequences;

d) cleaving the ligated sequences with one or more methylation-specific endonucleases, to produce amplifiable test nucleotide sequences, non-amplifiable test nucleotide sequences, amplifiable control nucleotides sequences and non-amplifiable control nucleotide sequences;

e) amplifying the amplifiable test nucleotide sequences and amplifiable control nucleotide sequences to produce amplified test nucleotide sequences and amplified control nucleotide sequences;

f) labelling the amplified test nucleotide sequences from step e) with a first label, and labelling the amplified control nucleotide sequence from step e) with a second label;

g) hybridising the labelled products of step f) with an array comprising a series of nucleotide sequences that are capable of hybridising thereto;

h) determining the ratio of the signals emitted by the first label relative to the second label for each hybridised nucleotide sequence on the array.

2) The method of claim 1, further comprising a step of correcting for the effect of DNA sequence variation:

i) amplifying the genomic test nucleotide sequences and separately amplifying the genomic control sequences with a DNA polymerase to produce an

unmethylated copy of the genomic test nucleotide sequences and an unmethylated copy of the genomic control sequences;

ii) treating the unmethylated copy of the genomic test nucleotide sequences and separately treating the unmethylated copy of the genomic control sequences with restriction endonuclease digestion, adaptor ligation, amplification, labelling, array hybridisation, and ratio determination steps that are equivalent to corresponding steps b), c) and e-h);

iii) comparing the one or more ratios determined in step j) to the one or more ratios determined in step h).

3) The method of claim 2, wherein the DNA polymerase of step I) is Phi29 DNA polymerase.

4) The method of claim 1 or 2, wherein the CpG specific endonuclease is McrBC.

5) The method of claim 1 or 2, wherein the methylation-sensitive restriction endonuclease is a cocktail comprising HpaII, Bsu151 (ClaI), HincII, AclI (SsiI), Tail, or any combination thereof.

6) The method of claim 1, wherein step f) further comprises labelling the non-amplified test nucleotide sequences from step d) with the first label, and labelling the non-amplified control nucleotide sequences from step d) with a second label.

7) The method of any one of claims 1 to 6, wherein the phenotype of interest comprises a disease such as cancer, diabetes, Alzheimer's disease, or schizophrenia, multiple sclerosis, psoriasis, atherosclerosis, asthma, autism, or rheumatoid arthritis.

8) The method of any one of claims 1 to 6, wherein said probe is a chemically reactive fluorophore.

9) The method of any one of claims 1 to 6, wherein said fluorophore is Cy 3 or Cy 5.

10) A method of analysing the methylation state of one or more nucleotide sequences comprising the steps of:

- a) selecting one or more genomic test nucleotide sequences from one or more subjects that exhibit a phenotype of interest and one or more corresponding genomic control sequences from one or more control subjects that lack the phenotype of interest;
- b) digesting the genomic test nucleotide sequences and separately digesting the genomic control sequences with one or more frequent cutting restriction endonucleases;
- c) ligating adaptor nucleotide sequences to the ends produced from step b) to produce ligated sequences;
- d) cleaving the ligated sequences with one or more methylation-sensitive restriction endonucleases to produce amplifiable test nucleotide sequences, non-amplifiable test nucleotide sequences, amplifiable control nucleotides sequences and non-amplifiable control nucleotide sequences;
- e) amplifying the amplifiable test nucleotide sequences and amplifiable control nucleotide sequences to produce amplified test nucleotide sequences and amplified control nucleotide sequences;
- f) labelling the amplified test nucleotide sequences from step e) with a first label, and labelling the amplified control nucleotide sequence from step e) with a second label;
- g) hybridising the labelled products of step f) with an array comprising a series of nucleotide sequences that are capable of hybridising thereto;
- h) determining the ratio of the signals emitted by the first label relative to the second label for each set of hybridised nucleotide sequence on the array.

11) The method of claim 10, further comprising a step of correcting for the effect of DNA sequence variation:

- i) amplifying the genomic test nucleotide sequences and separately amplifying the genomic control sequences with a DNA polymerase to produce an unmethylated copy of the genomic test nucleotide sequences and an unmethylated copy of the genomic control sequences;
- j) treating the unmethylated copy of the genomic test nucleotide sequences and separately treating the unmethylated copy of the genomic control sequences with restriction endonuclease digestion, adaptor ligation, amplification, labelling, array

- hybridisation, and ratio determination steps that are equivalent to corresponding steps b-h);
- k) comparing the one or more ratios determined in step j) to the one or more ratios determined in step h).
- 12) The method of claim 11, wherein the DNA polymerase of step i) is Phi29 DNA polymerase.
- 13) The method of claim 10 or 11, wherein the methylation-sensitive restriction endonuclease is a cocktail comprising HpaII, Bsu15I (ClaI), Hin6I, AclI (SsiI), Tail, or any combination thereof.
- 14) The method of claim 10 or 11, wherein the frequent cutting restriction endonuclease is selective for A/T rich sequences over C/G sequences.
- 15) The method of claim 14, wherein the frequent cutting restriction endonuclease comprises Csp6I, TasI, or a combination thereof.
- 16) The method of claim 10, wherein step f) further comprises labelling the non-amplified test nucleotide sequences from step d) with the first label, and labelling the non-amplified control nucleotide sequences from step d) with a second label.
- 17) The method of any one of claims 10 to 16, wherein the phenotype of interest comprises a disease such as cancer, diabetes, Alzheimer's disease, or schizophrenia, multiple sclerosis, psoriasis, atherosclerosis, asthma, autism, or rheumatoid arthritis.
- 18) The method of any one of claims 10 to 16, wherein said probe is a chemically reactive fluorophore.
- 19) The method of any one of claims 10 to 16, wherein said fluorophore is Cy 3 or Cy 5.
- 20) A kit comprising one or more genomic test nucleotide sequences, one or more corresponding genomic control nucleotide sequences, one or more frequent cutting

restriction endonucleases, one or more specific adaptor nucleotide sequences, one or more methylation-sensitive restriction endonucleases, one or more CpG specific restriction endonucleases, one or more probes for labelling the nucleotide sequences, one or more microarrays capable hybridising to the genomic test and control nucleotide sequences, software for displaying and/or analysing the sequences hybridised to the microarray, reagents and/or enzymes for amplifying nucleotide sequences, or any combination thereof.

21) A method of identifying DNA sequence variation in a methylation-state-analysis of one or more nucleotide sequences comprising the steps of:

- a) selecting one or more genomic test nucleotide sequence from one or more subjects that exhibit a phenotype of interest, for example a disease such as but not limited to cancer, diabetes, Alzheimer's disease, schizophrenia or the like, and one or more corresponding genomic control sequences from one or more control subjects that lack the phenotype of interest;
- b) amplifying the genomic test nucleotide sequences and separately amplifying the genomic control sequences with a DNA polymerase, for example without limitation a Phi29 DNA polymerase, to produce an unmethylated copy of the genomic test nucleotide sequences and an unmethylated copy of the genomic control sequences;
- c) treating the unmethylated copy of the genomic test nucleotide sequences and separately treating the unmethylated copy of the genomic control sequences with restriction endonuclease digestion, adaptor ligation, amplification, labelling, array hybridisation, and ratio determination steps that are equivalent to corresponding steps in the methylation-state-analysis;
- d) comparing the one or more ratios determined in step c) to the one or more ratios of the methylation-state-analysis, thereby identifying DNA sequence variation in the methylation-state-analysis.

22) A method of analysing the methylation state of one or more nucleotide sequences comprising the steps of:

- a) selecting one or more genomic test nucleotide sequences from one or more subjects that exhibit a phenotype of interest and one or more corresponding genomic control sequences from one or more control subjects that lack the phenotype of interest;
- b) digesting the genomic test nucleotide sequences and separately digesting the genomic control sequences with one or more methyl sensitive enzyme, followed by digestion of the genomic test nucleotide sequences and the genomic control sequences with a methyl-insensitive enzyme, the methyl insensitive enzyme being a neoschizomer of the corresponding methyl sensitive enzyme;
- c) ligating adaptor nucleotide sequences to the ends produced from step b) to produce ligated sequences;
- d) cleaving the ligated sequences with one or more methylation-sensitive restriction endonucleases to produce amplifiable test nucleotide sequences, non-amplifiable test nucleotide sequences, amplifiable control nucleotides sequences and non-amplifiable control nucleotide sequences;
- e) amplifying the amplifiable test nucleotide sequences and amplifiable control nucleotide sequences to produce amplified test nucleotide sequences and amplified control nucleotide sequences;
- f) labelling the amplified test nucleotide sequences from step e) with a first label, and labelling the amplified control nucleotide sequence from step e) with a second label;
- g) hybridising the labelled products of step f) with an array comprising a series of nucleotide sequences that are capable of hybridising thereto;
- h) determining the ratio of the signals emitted by the first label relative to the second label for each set of hybridised nucleotide sequence on the array.